

A NEW MEMBER OF THE *ENCHENOPA BINOTATA* SAY COMPLEX
ON TULIP TREE (*LIRIODENDRON TULIPIFERA*)

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Abstract.—A new North American member of the *Enchenopa binotata* Say complex is reported on tulip tree, *Liriodendron tulipifera* (Magnoliaceae). Electrophoretic analysis indicates that it is distinct from other members of the *Enchenopa* complex. The phenology of *Enchenopa* on tulip tree differs dramatically from that of most members of the complex, though eggs hatch and nymphs molt to adults at about the same time as *Enchenopa* on *Robinia pseudoacacia*. Mating and oviposition, however, appear to occur after that on *R. pseudoacacia*.

Enchenopa binotata Say is a complex of six reproductively isolated species in North America. Each of these biological species is restricted to a single species of host plant. The hosts are *Ptelea trifoliata* L. (Rutaceae), *Viburnum prunifolium* L. (Caprifoliaceae), *Celastrus scandens* L. (Celastraceae), *Juglans nigra* L. (Juglandaceae), *Cercis canadensis* L. (Fabaceae), and *Robinia pseudoacacia* L. (Fabaceae). Female *E. binotata* when given a choice will 1) mate on their original host, 2) mate with males from the same host and 3) select their original host for oviposition (Wood, 1980). Electrophoretic analyses have confirmed that *Enchenopa* from each host are distinct by the presence of unique alleles or in the frequencies of alleles. Electrophoretic patterns of *Enchenopa* from each host are consistent from locality to locality (Guttman et al., 1981).

Speciation in this complex appears to be the result of host plant shifts (Wood, 1980) similar to that proposed for *Rhagoletis* flies (Bush, 1969, 1975). Differing host phenologies and nutritional quality were postulated to promote allochronic maturation and mating (Wood, 1980). Allochronic mating combined with diurnal differences in mating effectively inhibits gene flow among the six *Enchenopa* species (Wood and Guttman, 1981, 1982). Reproductive success of mated females that were forced to oviposit on hosts other than their original host is low. Such females deposit fewer egg masses with lower viability than on normal hosts. Eggs hatch on "adopted" hosts at the same time as those from females native to that host. Thus the host plant appears to regulate when eggs hatch and establish allochronic life histories. Survival of nymphs on "adopted" hosts is extremely low (Wood and Guttman, 1983).

Here we report a new member of the *E. binotata* Say complex on tulip tree, *Liriodendron tulipifera* L. (Magnoliaceae). *Enchenopa* on this host have been found in Ohio (Clinton Co.) and Delaware (New Castle Co.). This new form may

Table 1 Allele frequencies at six polymorphic loci coding for enzymes in *Enchenopa binotata* from 7 host plants.

Locus	Allele	<i>Larodendron</i> N = 14	<i>Juglans</i> N = 150	<i>Ptelea</i> N = 158	<i>Cercis</i> N = 130	<i>Viburnum</i> N = 140	<i>Rubus</i> N = 26	<i>Celastrus</i> N = 158
<i>Est-1</i>	<i>a</i>					0.01		
	<i>b</i>	0.29	0.05	0.05	0.07	0.19		0.15
	<i>c</i>		0.67	0.58	0.07	0.29		0.41
	<i>d</i>	0.71	0.25	0.18	0.80	0.39	1.0	0.29
	<i>e</i>			0.19	0.08	0.10		0.10
	<i>f</i>					0.02		0.05
<i>Pep-2</i>	<i>a</i>		0.15	0.99				
	<i>b</i>	1.0	0.53	0.01	0.34			0.34
	<i>c</i>		0.30		0.64	0.98	1.0	0.66
	<i>d</i>		0.01		0.02	0.02		
<i>Pgi-2</i>	<i>a</i>		0.20	0.14		0.01		
	<i>b</i>	0.93	0.77	0.86	0.98	0.93	0.92	0.98
	<i>c</i>	0.07	0.01		0.02	0.06	0.08	0.01
	<i>d</i>		0.01					0.01
<i>Pgm-2</i>	<i>a</i>		0.01	0.14	0.02	0.02		0.01
	<i>b</i>					0.01		
	<i>c</i>	1.0	0.95	0.86	0.94	0.96	0.35	0.92
	<i>d</i>		0.03		0.02		0.04	0.03
	<i>e</i>		0.01		0.02		0.62	0.04
<i>Cat-1</i>	<i>a</i>	0.79	0.45		0.02	0.03	0.04	0.03
	<i>b</i>				0.34			
	<i>c</i>	0.21	0.46		0.62	0.22		0.25
	<i>d</i>		0.09	1.0	0.02	0.73	0.96	0.70
	<i>e</i>					0.02		0.01
<i>Got-1</i>	<i>a</i>							0.02
	<i>b</i>		0.03	0.08	0.08	0.03		0.19
	<i>c</i>	1.0		0.83	0.92	0.97	1.0	0.78
	<i>d</i>		0.97	0.09				0.01

N = number of genomes sampled.

Locus code: Est, esterase; Pep, peptidase; Pgi, phosphoglucose isomerase; Pgm, phosphoglucomutase; Cat, catalase; Got, glutamate oxaloacetate transaminase.

be geographically widespread but we have only found extremely isolated populations with low numbers of individuals; other members of the complex are generally abundant when found. Unlike other members of the complex, *Enchenopa* nymphs on tulip tree tend to be solitary or in small groups and have not been observed to be tended by ants. Nymphs after the first instar are green, making them difficult to locate when they are feeding in the leaf axils and apical meristem.

METHODS

Adult females were collected in August and September and forced to oviposit on branches covered with nylon netting. The following spring, branches with egg masses were observed daily and the number of nymphs counted. To compare the life history phenology of this *L. tulipifera* form to other members of the complex, similar data was collected from each of the six hosts. We treated the day that eggs hatched on the first host as day one and comparisons were made of the mean day eggs hatched on a given host (Wood and Guttman, 1982, 1983).

Table 2. Mean day *Enchenopa binotata* eggs hatched on 7 host species in 1983. Means with overlapping vertical lines represent no statistical differences (ANOVA and Duncan's Multiple Range Test).

Host Plant	Day Eggs Hatched
<i>V. prunifolium</i>	3.17
<i>C. scandens</i>	5.40
<i>P. trifoliata</i>	6.43
<i>J. nigra</i>	6.36
<i>C. canadensis</i>	10.04
<i>R. pseudoacacia</i>	12.84
<i>L. tulipifera</i>	18.83

The genetic relationships of *E. binotata* on tulip tree and the other six species were determined by starch gel electrophoresis of soluble proteins using the methods of Guttman et al. (1981). Six enzyme loci are polymorphic and consistently resolvable in *E. binotata*. Allele frequencies at these loci are listed in Table 1. Genetic similarity, distance coefficients and dendrograms were computed from frequencies using the Biosys-1 program of Swofford and Selander (1981).

RESULTS AND DISCUSSION

Oviposition by females begins in late August and continues through October in Delaware and Ohio. Eggs are deposited in branches representing the most recent growth and then covered with egg froth similar in appearance to that of *E. binotata* from *Viburnum*. Clumping of egg masses on branches is not as pronounced as with other members of the complex. Seldom have we found more than twelve egg masses on a branch while the usual number is five to six. The reduced number of egg masses on branches and the apparent greater dispersion within a host may be an artifact of low population densities on trees or may reflect differences in the biology of this form.

Eggs hatched in 1982 and 1983 about May 19 approximately 12 days after *E. binotata* eggs began to hatch on other hosts (Table 2). On mature host plants egg hatch occurred when *L. tulipifera* was in flower. First instars moved from the woody branch where eggs were deposited to feed on the apical meristem. As with other *Enchenopa*, nymphs at this stage are black. In succeeding instars, nymphs become green but continue to feed on petioles, apical meristems, and green shoots.

Table 3. Matrices of genetic distance (above diagonal) and genetic identity (below diagonal) (Nei, 1972) between *Enchenopa binotata* from 7 host plants.

Host Type	1	2	3	4	5	6	7
1. <i>Liriodendron</i>	*****	0.137	0.192	0.073	0.136	0.187	0.101
2. <i>Juglans</i>	0.872	*****	0.173	0.134	0.157	0.256	0.111
3. <i>Ptelea</i>	0.825	0.841	*****	0.161	0.098	0.159	0.078
4. <i>Cercis</i>	0.929	0.874	0.851	*****	0.052	0.099	0.048
5. <i>Viburnum</i>	0.873	0.855	0.906	0.949	*****	0.054	0.013
6. <i>Robinia</i>	0.829	0.774	0.853	0.906	0.948	*****	0.074
7. <i>Celastrus</i>	0.904	0.895	0.925	0.954	0.987	0.929	*****

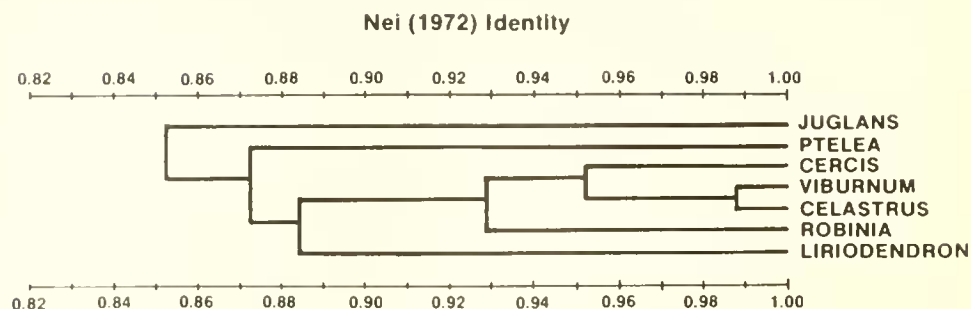


Fig. 1. Cluster analysis of *Enchenopa binotata* from seven hosts.

In 1983, nymphs began to mature on July 4, or 47 days after the first egg hatched. Eclosion to adults occurred over 10 days and began 8 days after that on *R. pseudoacacia*. *Enchenopa binotata* nymphs on *R. pseudoacacia* are always the last of the other members of the complex to turn adult. Because the number of *E. binotata* on *L. tulipifera* were low it is impossible to compare when mating occurred relative to other members of the complex. We did observe five matings and these took place when mating was almost over on all hosts except *R. pseudoacacia*.

Electrophoretically, *E. binotata* from *L. tulipifera* are distinct. Three (Pep-2, Pgm-1, Got-1) of the six loci were invariant in the seven insects examined from Clinton Co., Ohio and Delaware. One, three and four heterozygotes were found at the three remaining loci (Pgi-2, Cat-1, Est-1, respectively). The matrices of genetic distances and similarity coefficients (Table 3) indicate that while the *E. binotata* from *L. tulipifera* are uniformly distinct from those native to all other hosts they have their greatest affinity with treehoppers from *C. canadensis*. Cluster analysis (UPGMA), using Nei's genetic identity (I), indicates that *E. binotata* on *L. tulipifera* diverged after those on *P. trifoliata* but before those on *R. pseudoacacia* (Fig. 1).

Enchenopa binotata on *L. tulipifera* thus is a seventh member of the complex. Extremely limited observations suggest there may be an eighth on hickory, *Carya* (Juglandaceae). Adult *E. binotata* were reported on *Carya* by Funkhouser (1917) but until recently attempts to locate nymphs, adults and egg masses on this host have failed. The only *E. binotata* population on *Carya* we have been able to locate is in Clinton Co., Ohio. In a stand of four large *Carya*, one tree had *E. binotata* consistently over a 4 year period. Green nymphs were found on this tree feeding on the flower inflorescences next to developing nuts. Egg masses on this host were deposited below the apical bud in a fashion similar to those on *J. nigra*. Unfortunately the number of individuals on this host was low and our success in trying to build up a population on trees that were caged failed, so we did not compare the life history phenology nor electrophoretic patterns to other members of the complex.

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